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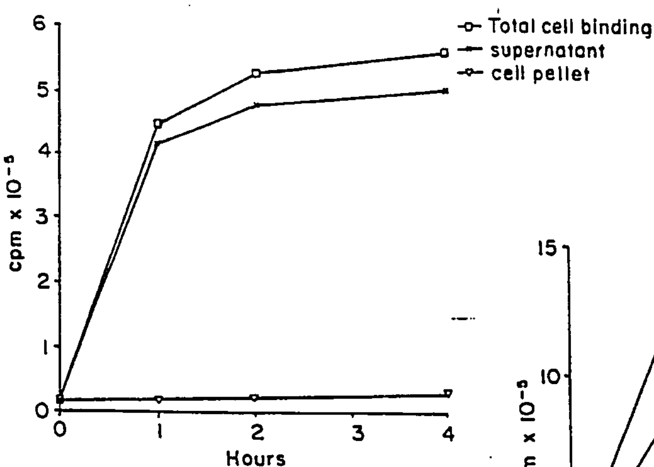

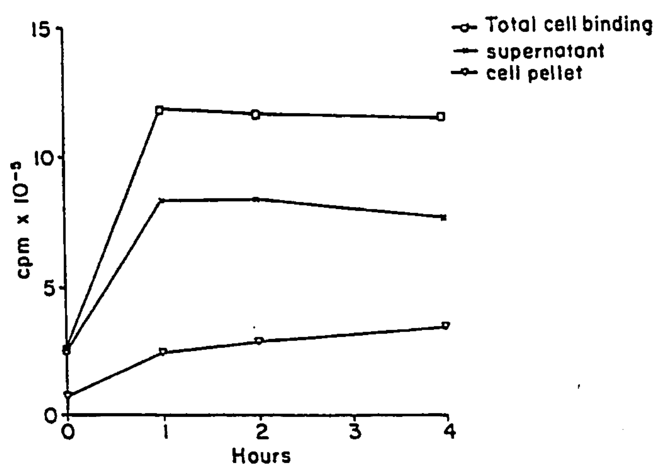

(57) Abstract

A radiotherapeutic immunoconjugate comprising a tumor specific monoclonal antibody, or fragment thereof, and an Auger electron emitting radionuclide, wherein the tumor specific monoclonal antibody or fragment is capable of tumor nucleus localization of the radionuclide is disclosed. The radiotherapeutic immunoconjugate is administered in a therapeutically effective amount to patients having or suspected of having a malignancy reactive with the antibody or fragment.

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TITLE

Radiotherapeutic Immunoconjugates Labeled with Iodine-125

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of therapeutic applications for tumor specific monoclonal antibodies, more particularly, to radiotherapeutic immunoconjugates useful for cancer therapy.

Background of the Invention

Immunotherapeutic techniques designed to localize radioisotopes proximate to cancer cells are being widely tested with a variety of radionuclides. In theory, such radionuclides could deliver sufficient radiation to destroy a tumor through numerous cell layers distant from the primary decay event. However, clinical studies indicate that radiolabelled monoclonal antibodies are capable of localizing less than about .004% of an injected dose of radioactivity to a human tumor. As a result it is uncertain whether monoclonal antibodies will be capable of delivering therapeutic quantities of radiation to a tumor volume without causing significant radiation toxicity to the remaining body.

Therapeutic applications of immunoconjugates of radioactive iodine in patients has been limited to the use of iodine-131 (^{131}I). Recently, studies relating to the _in vitro_ cytotoxicity of radioactive iodine-125 (^{125}I) labelled monoclonal antibodies have been reported in the technical literature. _Cancer Research_ _45_:5080 (1985) discloses specific killing of

human melanoma cells by a ^{125}I labelled murine monoclonal antibody directed against a M_r 250,000 melanoma-associated antigen. Blood 67:429 (1986) discloses selective-cytotoxicity of ^{125}I-labelled murine monoclonal antibody specific for the T65 antigen on human malignant T cell lines. The reference discloses that prolonged exposure times were achieved under frozen conditions and resulted in a low degree of internalization. Clinically useful applications for ^{125}I-labelled monoclonal antibodies are of signifigant interest to those in the biomedical field.

SUMMARY OF THE INVENTION

The present invention provides a radiotherapeutic immunoconjugate comprising a tumor specific monoclonal antibody, or fragment thereof, and an Auger electron emitting radionuclide, wherein the tumor specific monoclonal antibody or fragment is capable of tumor cell nucleus localization of the radionuclide. The radiotherapeutic immunoconjugate is administered in a therapeutically effective amount to patients having or suspected of having a malignacy reactive with the antibody or fragment.

In one embodiment, the invention provides a radiotherapeutic immunoconjugate comprising a monoclonal antibody, or fragment thereof, specific for the 17-1A antigen and ^{125}I. The invention further provides a method for treating 17-1A-positive malignancies comprising administering a therapeutically effective amount of the above radiotherapeutic immunoconjugate.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a shows internalization of ^{125}I MAb 17-1A in 17-1A-positive tumor cells (SW1116) following incubation at 0 °C. Figure 1b shows internalization following incubation at 37 °C.

Figure 2 shows internalization of ^{125}I MAb 17-1A in SW1116 cells following incubation at 37 °C.

Figure 3 shows the results of a control experiment wherin internalization of ^{125}I MAb 1116-NS-19-9 was measured in SW1116 cells following incubation at 0 °C and 37 °C.

Figures 4a, 4b, 4c, and 4d show chromosomal abberations scored in SW1116 cells due to specified ^{125}I labelled monoclonal antibodies and control agents.

Figure 5 shows number of cells with chromosome breaks (CB) per 100 metaphases after exposure to various concentrations of ^{125}I radiolabeled and unlabeled MAb 17-1A.

Figure 6 shows cell survival curve for SW1116 and 17-1A-negative tumor cells (WISH) following incubation with ^{125}I radiolabelled MAb 17-1A.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a cancer therapy method which employs an immunoconjugate comprising a tumor specific monoclonal antibody, or fragment thereof, and an Auger electron emitting radionuclide, wherein said tumor specific monoclonal antibody or fragment is capable of tumor cell nucleus localization. As used herein, the expression "tumor cell nucleus localization" means that the antibody or fragment demonstrates specific incorporation into the nucleus of mammalian cells and its related components (nuclear membrane, nucleoplasm, chromatin, chromosomes, nucleolus, nucleosomes, DNA and RNA). It has been found that the immunoconjugates of the invention is capable of selective cytotoxity for tumor without harming normal tissues.

Tumor specific monoclonal antibodies useful in the invention can be obtained by immunizing an animal with a biological sample containing tumor associated antigen, forming a fused cell hybrid between antibody producing cells from the animal and myeloma cells, cloning the resulting hybrid and selecting clones which produce antibodies that bind to the tumor associated antigen. Techniques for immunizing animals, forming fused cell hybrids, and selecting clones with the desired binding specificity are known in the art. The preparation of antibody fragments from intact antibodies are also well know to those skilled in the field of immunology. Preferably, a biological sample containing the 17-1A antigen is employed to immunize an animal and a monoclonal antibody, or fragment thereof, having specificity to the 17-1A antigen is employed in the invention.

The monoclonal antibody 17-1A (herein MAb 17-1A) has been reported to bind to a tumor specific antigen (herein "17-1A antigen") associated with gastrointestinal adenocarcinomas (Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D. & Fuhrer, J.P. Somat. Cell Genet., 5:957-972 (1979); Sears, H.F., Herlyn,D., Herlyn, M., Grotzinger, P.J., Steplewski, Z., Gerhard, W. & Koprowski, H. J. Surg. Res., 31:145-150 (1981)). The antigen has been characterized (Ross, A.H., Herlyn, D. Iliopoulos, D., and Koprowski, H. Biochemical and Biophys Res Comm., 135:297-303 (1986)) and is described as a cell surface protein with two subunits of 30,000 to 40,000 daltons.

Auger electron emitting radionuclides are known in the art. For example, radioactive iodine-125 decays by electron capture ($T_{1/2}$ = 60.5 days) to the metastable state of tellurium-125m which undergoes immediate decays events by internal conversion (93%) and by gamma ray emission (7%) to the tellurium-125 daughter. Due to inner shell vacancies produced, a complex series of electron shell rearrangements occur resulting in low energy x-rays (27-35 keV) and a cascade of low energy electrons.

Tumor specific monoclonal antibodies, or fragments thereof, can be labelled with an Auger electron emitting radionuclide by any of the many known labelling methods. The preferred technique for ^{125}I labeling is that of Markwell, M.A.K. & Fox, C.F. Biochemistry 17:4807-4817 (1978), the teaching of which is incorporated herein by reference. Antibodies can also be labelled with radioiodine by a variety of other well known procedures (eg. Chloramine T, Bolton-Hunter, Lactoperoxidase, etc).

In the method of the invention, the radiotherapeutic immunoconjugate is administered in a therapeutically effective amount to a patient having or suspected of having a malignacy reactive with the antibody or fragment component of the immunoconjugate. Methods for administrating radiotherapeutic immunoconjugates to patients are known in the art. Preferably, the present immunoconjugate is administered in an amount of from about 20 to 200 milliCuries of radioactivity representing mass quantities of antibody of about 2 to 20 mg.

Although not wishing to be bound by theory, it is believed that in order for the present ^{125}I labeled monoclonal antibody to be effective, the monoclonal antibody must first be internalized significantly into the cell as a result of binding to its specific membrane antigen. It is further believed that for maximum cell killing of 17-1A positive malignancy, the I-125 radiolabeled monoclonal antibody or its radioactive breakdown products must bind directly to the nucleus.

The invention is further described by the following examples wherein all parts and percentages are by weight and degrees are Celsius.

EXAMPLES

MATERIALS AND METHODS

Tumor cell lines and monoclonal antibodies. Human colon cancer SW1116 cells were cultured in RPMI 1640 medium supplemented with 20% fetal calf serum. Another cell population designated WISH, human amnion cells, obtained from the American Type Tissue Collection (Rockville, Md) was cultured in the same

medium containing 10% fetal calf serum. This cell line contained no tumor specific antigens that binds the MAb 17-1A. The cell lines were maintained in a humidified incubator with an atmosphere of 5% CO_2 in air at 37 ^{o}. For the following examples and control experiments, all cells in log phase were harvested by removing the medium, washing with 0.05% trypsin containing 0.02% EDTA and 5 ug/mL DNAase and resuspended in fresh medium to various cell concentrations depending upon the study. The monoclonal antibodies 17-1A, IgG(2a) isotype; 1116-NS-19-9, IgG(2a) isotype; and R11D10, IgG(2a) isotype were employed in the assays. The 1116-NS-19-9 monoclonal antibody binds to a colon cancer antigen that is shed from the SW1116 cell line. The R11D10 monoclonal antibody demonstrates reactivity with cardiac myosin and was used as an nonimmunoreactive control antibody.

Radioiodination. All monoclonal antibodies were labeled with ^{125}I using the Iodogen^{TM} (Pierce Chemical Co.) method (Markwell, M.A.K. & Fox, C.F. Biochemistry 17:4807-4817 (1978)). To Iodogen coated tubes, 200 ug of antibody (10 mg/mL, Albumin free) were added followed by 80 uL of PBS (0.1M phosphate buffer in saline, pH 7.0). Approximately 2.5 milliCuries of $Na^{125}I$ (carrier free, Dupont NEN) were immediately added and the resulting reaction was carried out for 10 minutes. The reaction was quenched using an ascorbic acid solution (.5 mL, 10 mg/mL) and the bound ^{125}I separated from any free iodide ion on a Sephadex G-25M column (Pharmacia, Uppsala Sweden) which was prewashed with a 2% Human serum albumin saline solution. The radiolabeled antibody was eluted in the void volume and assayed in a sodium iodide gamma scintillation counter.

Immunoreactivity and Internalization. Immunoreactivity of the ^{125}I labeled MAb 17-1A was evaluated by direct cell binding with various concentrations of radioactive antibody. SW1116 cells (5×10^5) were incubated in 100 ul of RPMI 1640 medium (20% fetal calf serum) at 37 ^{o} for 4 hours in microfuge tubes (Beckman) with various concentrations of labeled antibody (.039 - 20 uCi/mL). After incubation, the cells were centrifuged and washed twice with PBS (pH 7.4) containing 10% horse serum (Flow Laboratories, Va) and 0.02% NaN_3. The final cell pellet was counted in a sodium iodide gamma scintillation counter set to detect the 35 keV x-rays of ^{125}I.

The internalization of ^{125}I labeled MAb 17-1A was determined by incubating 2×10^6 cells in RPMI 1640 medium (20% fetal calf serum) in a fixed concentration of antibody (0.5 uCi/mL, .72 ug, 10 uL) for various time periods (from 0 to 48 hours). After incubation at 37 ^{o} with occasional shaking, the mixture was centrifuged and washed four times with 1 mL of the PBS containing 10% horse serum and 0.02% NaN_3. After the final centrifugation, the supernatants were discarded and the cell pellet assayed for radioactivity. The total cell binding (cell-associated) radioactivity was determined. The cell pellets were then resuspended in 0.2 mL of glycine-HCl buffer, pH 2.8, and incubated for 20 minutes at 0 ^{o}. The total cell-associated radioactivity was separated by centrifugation into the cell pellets which contained the acid-unremovable portion and the supernatants which contained the acid-removable portion of the radioactivities. The supernatants and the cell pellets were separately assayed for their

radioactive content. This procedure was then repeated with the incubation periods done at 0 ^{o}.

Assays for Chromosomal Damage. Approximately 10^6 cells in log phase were inoculated into a flask (25 cm^2) containing 5 mL of growth medium. After incubation at 37 ^{o} for 24 hours, various concentrations of ^{125}I labeled monoclonal antibodies or control agents were added to the culture and the resulting mixtures were further incubated for 48 hours. The cells were arrested in metaphase with demecolcine (0.06 ug/mL) 5 hours prior to termination of the incubation. The monolayers were dispersed by incubation with 0.05% trypsin for 10 minutes and the cells treated with 10 mL of 0.075 M KCl as a hypotonic for 40 minutes at 37 ^{o}. The resulting cell suspension was transferred to 15 mL centrifuge tubes and gently mixed with 5 mL of freshly prepared Carnoy's solution (acetic acid:methanol, 1:3) and incubated at ambient temperature for 10 minutes. The cells were harvested by centrifuging at 1000 rpm for 10 minutes, removing the supernatant, and gently resuspending in 10 mL of fresh Carnoy's solution. The incubation period at ambient temperature, centrifugation, and resuspension were repeated three times. Finally 3-4 drops of cell suspension were dropped onto clean, cold wet slides and allowed to air dry. The slides were then stained with Giemsa. A total of 100 cells from duplicate experiments (50 cells from each) were analyzed at each dose level under oil immersion. They were scored for chromatid breaks, acentric fragments, multicentric chromosomes, rings, and chromatid exchanges.

In the quantitative analysis, the various types of chromosomal abberrations were expressed as chromosome breaks (CB). Fragment and chromatid breaks were assigned one break; dicentric and ring figures were scored as two breaks. In instances of chromatid exchange, the number of breaks were determined by the configuration and number of chromosomes involved in the exchange figure.

For the determination of micronuclei (MN), procedures for cell culture and the ^{125}I antibody treatments were the same as the chromosome assay. After trypsinization and swelling in hypotonic KCl for 10 minutes at 37 ^{o}, the single-cell suspensions were fixed in ice-cold Carnoy's fixative, and were dropped onto clean glass slides. Cytoplasmic structures were scored as MN if they showed the same Giemsa staining reaction as the nucleus, were clearly resolved from the nucleus (to distinguish them from nuclear blebs), and had diameters that were 1/6 to 1/3 of the nucleus. A total of 2000 cells from duplicate experiments (1000 from each) were scored and averaged for each data point.

Cell Survival. Exponentially growing cells were trypsinized and serial dilutions of cell suspensions were prepared. An appropriate number of viable single cells ($1x10^3$ - $4x10^4$), depending upon the expected plating efficiency and surviving fraction, were seeded onto 3-5 , 60 mm petri dishes containing 4 mL of growth medium. The different concentrations of ^{125}I labeled MAb 17-1A (0-40 uCi/mL) and control agents were added at the same time. The cells were incubated at 37 ^{o} for 48 hours. The doubling time for the SW1116 cells

was determined to be about 48 hours (direct cell counting by typan blue exclusion of known cell numbers at various time intervals). After exposure for 48 hours with the test agents, the medium was aspirated and cells washed once with PBS. New growth medium was added and the cells incubated for another 19 days in the humidified 5% CO_2 atmosphere at 37 o to allow for colony formation. Plates were then stained with Crystal Violet (Fisher Scientific Co.) and individual colonies containing more than 50 cells were counted. The cell survival value was determined using the following formula.

$$\text{fractional survival} = \frac{\text{mean plate count of tests}}{\text{number of cells plated} \times \text{P.E.}}$$

$$\text{P.E. (plating efficiency)} = \frac{\text{mean plate count of controls}}{\text{number of cells plated}}$$

Duplicate independent experiments were used to determine the average fractional survival. A 17-1A-negative tumor cell line (WISH) was also used for cell survival with the radiolabeled MAb 17-1A.

RESULTS

The radiolabeling of MAb 17-1A with ^{125}I resulted in a specific activity of 10-12 mCi/mg. The average radioiodination yields were 90 - 95% efficient. This procedure can be scaled up to radiolabel much larger quantities of I-125, greater than 25 milliCuries. The immunoreactivity of the final labeled product was evaluated in a simple cell binding assay (Figure 1) which indicates the relative amount of radioactivity bound to tumor cells (SW1116) exposed to various concentrations of ^{125}I labeled antibody compared to an nonimmunoreactive ^{125}I antibody (R11D10) which showed negligible binding (not graphed due to being off scale). The amount of radioactivity bound by cells exposed to MAb 17-1A becomes a constant fraction when the radioactive concentration is greater than 10 uCi/mL. For the number of cells (2×10^5) used in this assay, 0.67 pCi of ^{125}I appear maximally bound per cell or 1.5 dpm/cell. This amounts to approximately 1.9×10^5 radioiodine atoms per cell or .047 picograms of antibody (assuming 1 iodine atom per antibody molecule). The radiolabeled nonimmunoreactive MAb R11D10 showed little total cell binding to the SW1116 cells. (about 2.5 x 10-4 dpm per cell).

The internalization of ^{125}I labelled MAb 17-1A by SW1116 cells was determined by measuring the cell binding at either 0 or 37 ^{0}, and using a mild acid wash to disassociate the antigen-antibody complex which released free antibody from membrane bound antigen back into solution. In these

examples and experiments, the following formula was employed:

% internalization = radioactive counts in cell pellet (acid-unremovable) x 100%/total cell binding counts.

The amount of internalized I-125 radioactivity after cells were exposed to ^{125}I labelled MAb 17-1A was determined and the results are shown in Figure 1a and 1b. As shown in Figure 1a, the total cell binding curve (cell associated radioactivity) and the supernatant curve (acid-removable radioactivity) are similar at 0 ^{0} since greater than 90% of the antibody was disassociated from membrane bound antigen and recovered in the supernatant at the various time periods. Incubation of SW1116 cells at 37^{0}C (Figure 1b) with the radioactive MAb 17-1A resulted in a significant portion of radioactivity remaining with the cell pellet (acid-unremovable) which increased with time of incubation leaving less radioactivity in the supernatant (acid-removable).

The relative percentage internalized into the SW1116 cell increased signficantly from 4 hours (29%) to 48 hours (49%) incubation as shown in Figure 2. Therefore, these results suggest that internalization of the radioiodinated MAb 17-1A occurrs after binding to its cell specific antigen and that amount internalized increases over time. Results obtained from studies with the radiolabelled (^{125}I) nonimmunoreactive R11D10 antibody (458 - 695 cpm, 0-4 hours at 37 ^{0}; 372 - 491cpm, 0-4 hours at 37 ^{0}) and ^{125}I labelled 1116-NS-19-9 antibody are shown in Figure 3 and suggested that little or no internalization took place at 37 ^{0}.

Chromosomal abberations in mitotic figures of cells exposed to increasing concentrations of radiolabeled ^{125}I monoclonal antibodies and control agents were scored and the results are shown in Figures 4a, 4b, 4c, and 4d. In these experiments, the number of cells per 100 metaphases with chromosome breaks (CB) were scored from duplicate samples which varied ± 10% and the results are shown in Figure 4a. (Zero uC/ml concentrations represents background chromosomal abberations which were determined at the time with each test agent.) In each sample, 50 cell metaphases were analyzed. The total number of CB in 100 metaphases are shown in Figure 4b. The results demonstrated that multiple breaks occur at higher radioactive concentrations. The number of cells with micronuclei (MN) formed per 1000 cells were also scored and the results (average of duplicate samples which varied ± 10%) are shown in Figure 4c. The average total number of MN per 1000 cells from duplicate samples (± 10% variation) are shown in Figure 4d.

Figures 4a-d indicate specific nuclear damage by the ^{125}I decay events. The ^{125}I-R11D10 and $Na^{125}I$ at increasing radioactive concentrations did not result in any increased frequency of abberations or total number of abberations. The noninternalized ^{125}I labelled 116-NS-19-9 which binds a shed antigen on the SW1116 cells resulted in slightly greater frequency of chromosome abberations than normal control values. The Auger electron damage caused by the ^{125}I labelled MAb 17-1A demonstrated a dose dependent response related increase in chromsomal abberations as seen with increased frequency and total number of abberations scored (Figures 4a and 4b).

Chromosomal studies with a equivalent mass amounts of radioactive and nonradioactive MAb 17-1A at constant specific activity were performed in order to determine whether the cellular damage was due to ^{125}I alone. In this experiment, numerical values were scored from duplicate samples (50 cell metaphases) with ± 10% variation. Figure 5a shows chrosomal breaks in SW1116 versus WISH cell lines exposed to ^{125}I labelled MAb 17-1A. Figure 5b shows the results when radiolabeled MAb 17-1A is compared to equilivant molar concentration of unlabeled MAb 17-1A in SW1116 cells and indicates no increased chromosomal damage above normal background levels for unlabelled antibody.

Chromosomal damage was not observed to any significant degree in the WISH cell line exposed to ^{125}I labeled MAb 17-1A as shown in Figure 5a. Another indication of specific nuclear damage caused by radiation effect is the formation of micronuclei in cells. The mean frequency and total mean number of micronuclei per 1000 cells (mean value of 2 separate experiments) was significantly increased above control agents as shown in Figures 4c and 4d. These results suggest that the ^{125}I labeled MAb 17-1A can induce specific chromosomal damage, presumably due to the Auger electrons, that increases proportionally with increasing radioactive concentration. At the higher radioactive concentrations (greater than 10 uCi/ml), there is a greater number of overall chromosome breaks (CB) due to increased multiple breaks per cell as well as an increased number of micronuclei (MN). In addition, there was about a ten-fold increase in the numbers of cells showing characteristic radiation induced pulverization of the chromosomes at the higher radioactive

concentrations. The results suggest that specific chromosomal damage can be caused by an Auger electron emitter attached to a monoclonal antibody which is internalized and interacts directly with the DNA.

The effect of ^{125}I labeled MAb 17-1A on cell survival was determined using SW11116 and WISH cell lines and the results are shown in Figure 6. In these experiments, log (% fractional survival) was measured against radioactive concentration. Numerical results were based on the average of duplicate experiments. ^{125}I labelled R11D10 as nonimmunoreactive antibody was compared to ^{125}I labelled MAb 17-1A in SW1116 cells. In addition, ^{125}I labelled MAb 17-1A cell survival in WISH cells was compared to unlabeled MAb 17-1A at equilivant molar concentrations (1.4 - 5.6 x 10^{-9} M).

The cell survival curve for ^{125}I labelled MAb 17-1A indicates a 3 log reduction in colony counts (Figure 7) as a function of increasing radioactive concentration (2.5 to 40 uCi/ml). The ^{125}I labelled MAb 17-1A demonstrated no cytotoxicity on the WISH cell line and exposure of SW1116 cells to increasing nonradioactive MAb 17-1A concentration (1.4-5.6 x 10-9 M) had no effect on colony survival. Also, the effect of radiolabeled ^{125}I-R11D10 (nonimmunoreactive antibody) had no effect on cell survival. These results suggest that the ^{125}I is responsible for causing decreased survival of cells containing antigen specific to the radiolabeled MAb 17-1A since cells which do not contain antigen are not affected by the ^{125}I.

DISCUSSION

It has been found that exposure of 17-1A-positive tumor cells with an immunoconjugate comprising a monoclonal antibody specific for the 17-1A antigen and ^{125}I can result in lethal effects. It is believed that the first requirement for such lethal effects to occurr is that the antigen-antibody complex undergo internalization into the cell. The second requirement is believed to be ^{125}I binding to the nucleus of the tumor cell whereby the maximum effect due to the subcellular range of Auger electrons is achieved. The radiation damage to the cell is ultimately due to chromosomal damage in which much of the existing data strongly indicates that ^{125}I when incorporated into DNA can result in irreparable damage and efficient cell killing.

The foregoing studies indicate that radiolabeled MAb 17-1A meets the first requirement by becoming significantly internalized after binding to specific cell membrane associated antigen. The internalization of ^{125}I labelled MAb 17-1A results in 29.7% of total cell associated radioactivity being incorporated into the cell at 4 hours which increased to 49% at 48 hours. During these time periods, no dehalogenation or release of iodine back into the extracullar medium has been observed using standard chromatography methods to detect any free iodide ion in the extracellular fluids and supernatants (after centrifugation and washing) during the incubation periods.

The maximum binding of ^{125}I labeled MAb 17-1A in SW1116 cells appears to plateau at around 10 uCi/ml concentration. The amount of radioactivity calculated becomes constant at about .67 pCi/cell. Assuming that 29 to 49% of total radioactivity bound to the cell is internalized, then 0.19 to .33 pCi is associated with the intracellular volume of the cell. This value is approximately 5 to 10 times higher than the uptake per cell of ^{125}I-iododeoxyuridine (.035 pCi/cell) required to achieve a 37% survival in V79 Chinese hamster cells. However, in our experiments the fraction of radioactivity internalized that becomes associated with the nucleus has not been determined and could be significantly less, but still be more than adequate to produce lethal consequences in the affected cells if associated directly with the nucleus and DNA.

The nuclear damage associated with ^{125}I incorporated into the nucleus results in characteristic radiation damage of the chromosomes. This damage manifests itself as the number of chromosome breaks (CB) as well as micronuclei (MN) and is proportional to the radioactive concentration in the nucleus. The foregoing studies show a similar dose dependent response related to increased chromosomal damage due to incorporation of radiolabeled MAb 17-1A which is specific to only tumor cells containing the requisite antigen. Control agents such as $Na^{125}I$, ^{125}I labelled R11D10 antibody, and ^{125}I labelled 116-NS-19-9 antibody did not result in an appreciable increase in chromosomal damage at similar radioactive concentrations in the SW1116 cell line. However, radioactive 116-NS-19-9 did show a slight increase in chromosomal abberations that does not appear to be dose dependent. Since the

116-NS-19-9 monoclonal antibody does bind to another tumor associated antigen on the SW11116 cell line which is shed into the extracellular medium, it is possible that the antigen-antibody complex may be taken up into these cells during the incubation period due to phagocytotic activity rather than direct internalization. Our studies further indicated that radioactive 116-NS-19-9 does not appear to be internalized as shown in Figure 3. Furthermore, it appears that unless the specific antigen is present on the tumor cell, cells which do not contain the antigen (WISH cell line) are not affected by the radiolabeled tumor specific antibody and no increase in chromosomal abberations are observed above normal background levels.

Regardless on the cell system used, chromosomal damage induced by radiation is highly correlated to increased lethality resulting in a diminished cell survival. The cytoxicity of ^{125}I labeled MAb 17-1A was determined using cell survival data. Nonimmunoreactive radiolabeled antibody and cells containing no antigen are not affected by the radioiodinated MAb 17-1A. The nonantigenic WISH cell line used for these studies have similar radiation sensitivities to the SW1116 cell line based upon cell survival studies using photon irradiation. However, because ^{125}I Auger electron lethality is by the direct effect as with high LET radiation, varying degrees of radiation sensitivities by x-ray photons would probably have little or no effect on the direct action of Auger electrons in the DNA. The length of time for the incubations of cells with the radioactive test agents were 48 hours. When incubations were carried out for only 4 hours, no affect on cell survival was observed with the SW1116 cell line and ^{125}I labelled MAb 17-1A.

This factor may be attributable to the amount of internalization of the radioactive antibody which increased with time. The affect of an internalized tumor specific monoclonal antibody labeled with ^{125}I on cell survival appears to be dose dependent on the amount of ^{125}I that is retained in the cell.

In comparison to the internalization of $^{125}IUdR$ in V79 cells, nuclear uptake is solely dependent upon mitotic activity while with a radiolabeled monoclonal antibody, other factors important for internalizing and nuclear binding may be involved and not necessarily be related only to cellular division. The survival curve obtained for ^{125}I labelled MAb 17-1A in SW1116 cells appears to have a slight shoulder which suggests that the ^{125}I lethality may not be similar to high LET radiation as has been suggested for $^{125}IUdR$ when incorporated into DNA. However, the radiation induced damamge of 125I Auger electrons may be modified in the presence of oxygen suggesting that secondary mechanisms which generate reactive free radicals may be also responsible for cellular damage. In addition, the highly lethal damage of ^{125}I to the DNA may be resultant of the large positively charged ^{125}Te daughter atom (+8) which may cause direct chemical changes within biologically sensitive molecules.

These studies reflect the critical importance of specific cellular binding, internalization, and nuclear interaction, for promoting individual tumor cell cytotoxicity using certain ^{125}I radiolabeled monoclonal antibodies such as MAb 17-1A. The primary mechanism of cell killing is understood in general terms of highly localized energy deposition within the DNA.

Theoretical and experimental arguments for the apparent high LET toxicity of ^{125}I support the notion of electron energy deposition (315 eV) of Auger electrons within a sphere of 10-Angstrom radius around the decay site within the DNA. The expected energy density has be postulated to be equivalent to 1.3×10^9 rads per decay. Therefore, it has been predicted that a short segment of DNA corresponding to about three base pairs long on either side of the decay event should be severely affected resulting in multiple strand breaks and base damage.

The ability of tumor specific ^{125}I labeled monoclonal antibodies to cause specific tumor cell destruction in vivo offers a distinct advantage for radioimmunotherapy. Radioiodination handling procedures and techniques are relatively simple, safe (low radiation exposure) and economic to perform yielding high specific activity product. Radioiodine-125 exposure to nontumor cells does not appear to cause any harm, and certainly whole body exposure from the weak iodine x-rays probably will not be of major concern when treating patients with large dosages of radioactive iodine. Other radionuclides such as ^{131}I, ^{90}Y, and alpha emitters have substantially higher particulate radiations and have the capability to destroy significantly more than just the tumor cells, thereby causing potentially more radiotoxicity. It is believed that the ability of circulating ^{125}I labeled monoclonal antibodies to destroy individual tumor cells without harming nontumor cells will make such therapy ideal candidates as adjuvants to standard cancer treatments should there be a likelihood for recurrence or metastatic disease.

CLAIMS

What is claimed is:

1. A radiotherapeutic immunoconjugate comprising a tumor specific monoclonal antibody, or fragment thereof, and an Auger electron emitting radionuclide, wherein the tumor specific monoclonal antibody or fragment is capable of tumor nucleus localization of the radionuclide.

2. A radiotherapeutic immunoconjugate comprising a monoclonal antibody, or fragment thereof, specific for the 17-1A antigen and ^{125}I.

3. A radiotherapeutic immunoconjugate comprising a MAb 17-1A and ^{125}I.

4. A method for treating a patient having a malignancy comprising administering a therapeutically effective amount of the radiotherapeutic immunoconjugate of Claim 1 wherein the antibody or fragment is reactive with the malignancy.

5. A method for treating a patient having a 17-1A-positive malignancy comprising administering a therapeutically effective amount of the radiotherapeutic immunoconjugate of Claim 2.

6. A method according to Claim 5, wherein the radiotherapeutic immunoconjugate is administered in an amount of from about 20 to 200

milliCuries of radioactivity representing mass quantities of antibody of about 2 to 20 mg.

7. A method for treating a patient having a 17-1A-positive malignancy comprising administering a therapeutically effective amount of the radiotherapeutic immunoconjugate of Claim 3.

8. A method according to Claim 7, wherein the radiotherapeutic immunoconjugate is administered in an amount of from about 20 to 200 milliCuries of radioactivity representing mass quantities of antibody of about 2 to 20 mg.

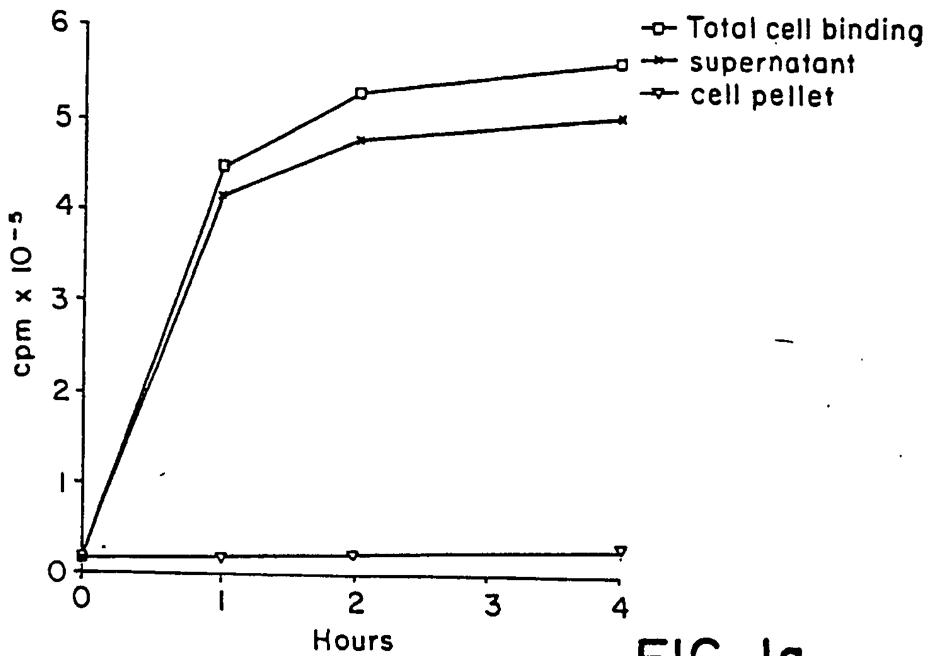

Total cell binding
supernatant
cell pellet
cpm x 10⁻⁵
0
1
2
3
4
5
6
Hours

FIG. 1a

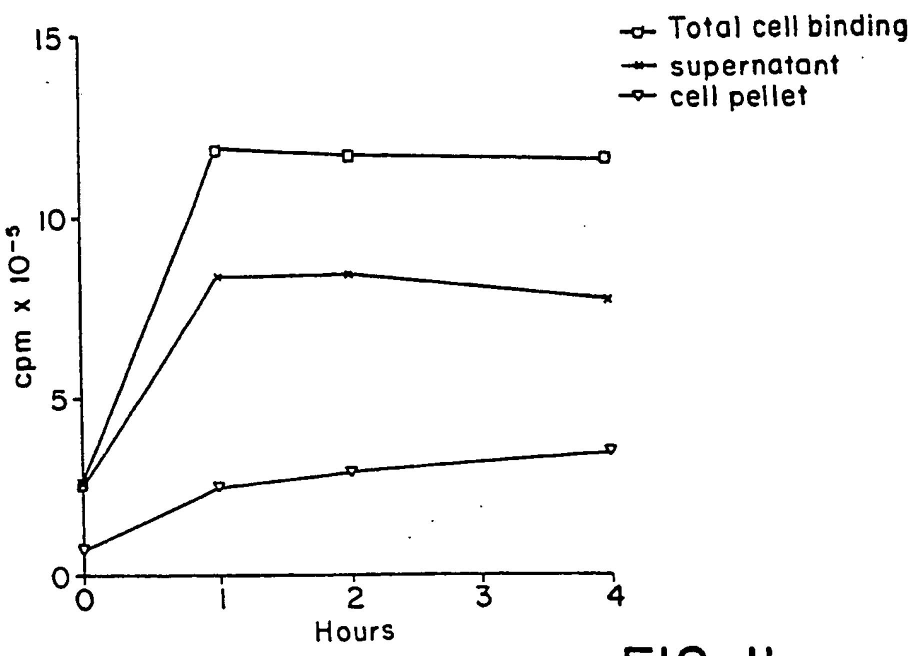

Total cell binding
supernatant
cell pellet
cpm x 10⁻⁵
0
5
10
15
1
2
3
4
Hours

FIG. 1b

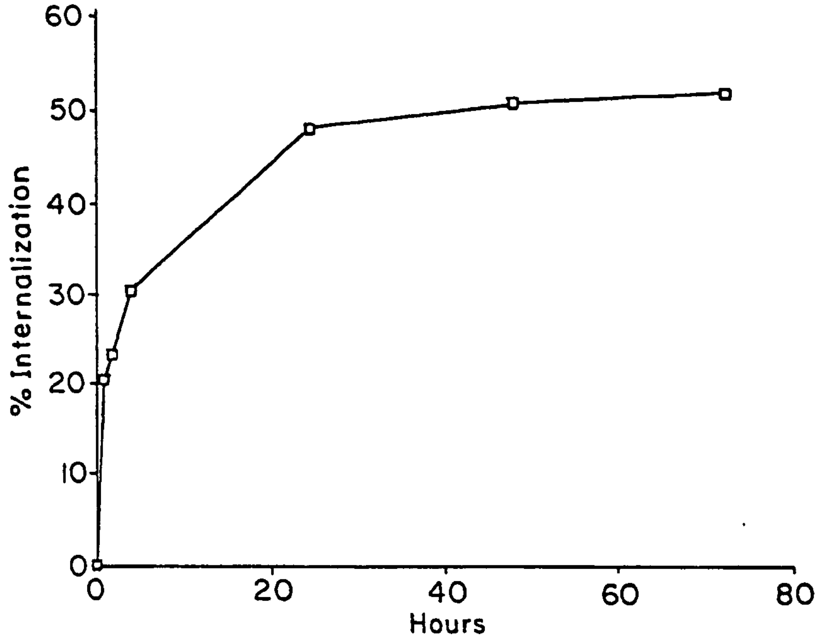

60
50
40
30
20
10
0
% Internalization
0
20
40
60
80
Hours

FIG. 2

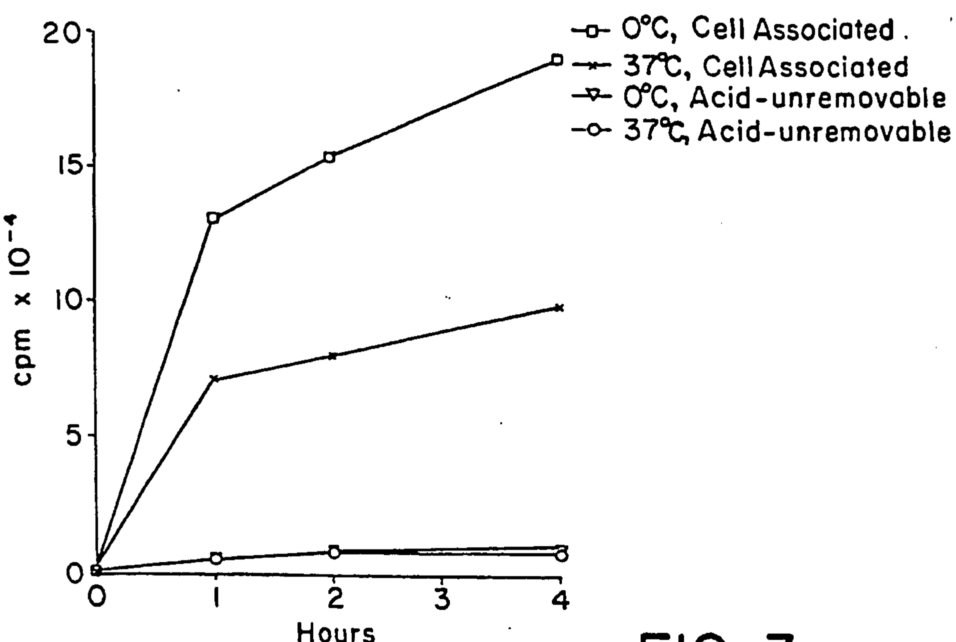

0°C, Cell Associated
37°C, Cell Associated
0°C, Acid-unremovable
37°C, Acid-unremovable
20
15
10
5
0
cpm x 10⁻⁴
0
1
2
3
4
Hours

FIG. 3

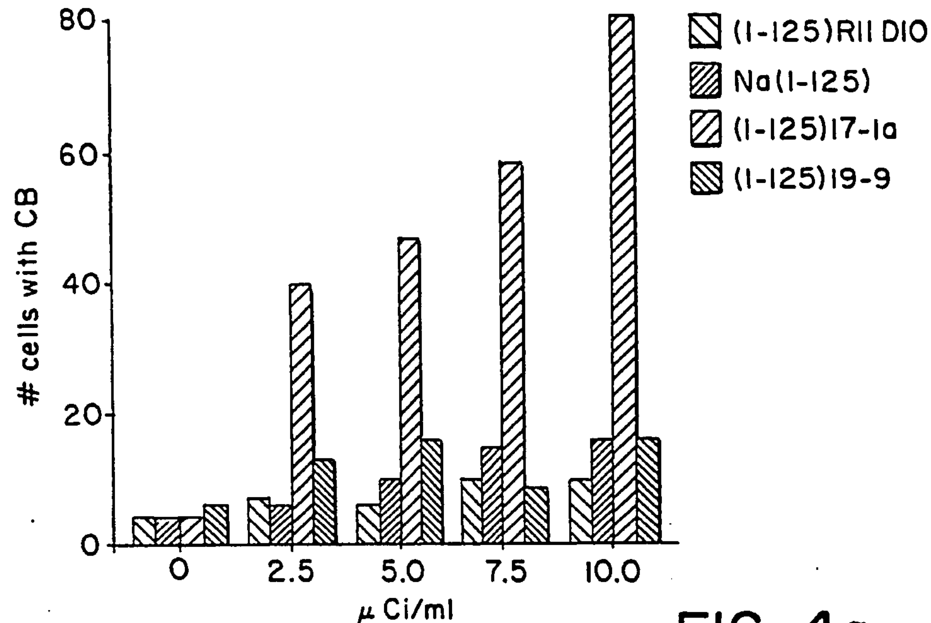

80
60
40
20
0
cells with CB
0
2.5
5.0
7.5
10.0
μ Ci/ml
(I-125)RII DIO
Na(I-125)
(I-125)17-Ia
(I-125)19-9

FIG. 4a

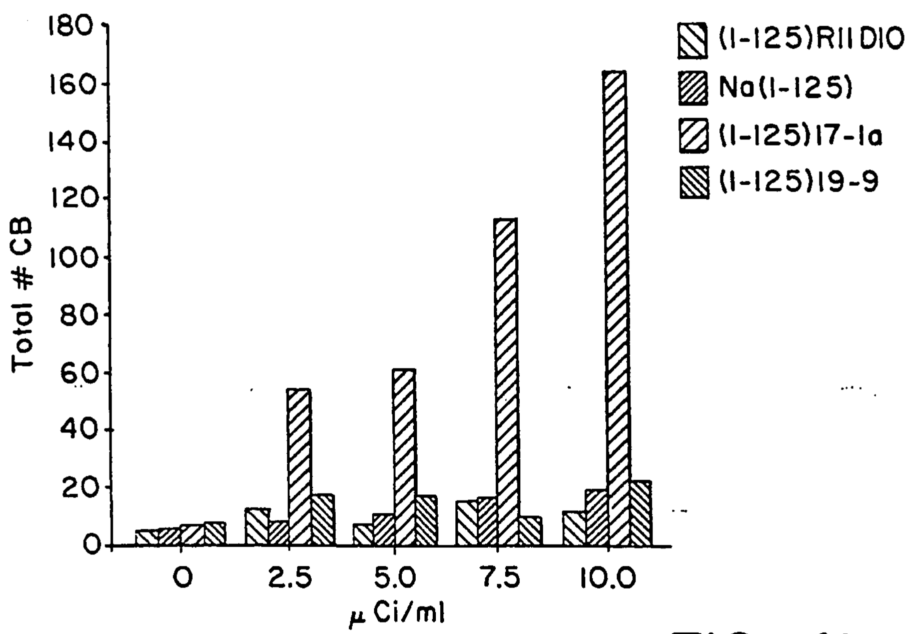

180
160
140
120
100
80
60
40
20
0
Total # CB
0
2.5
5.0
7.5
10.0
μ Ci/ml
(I-125)RII DIO
Na(I-125)
(I-125)17-Ia
(I-125)19-9

FIG. 4b

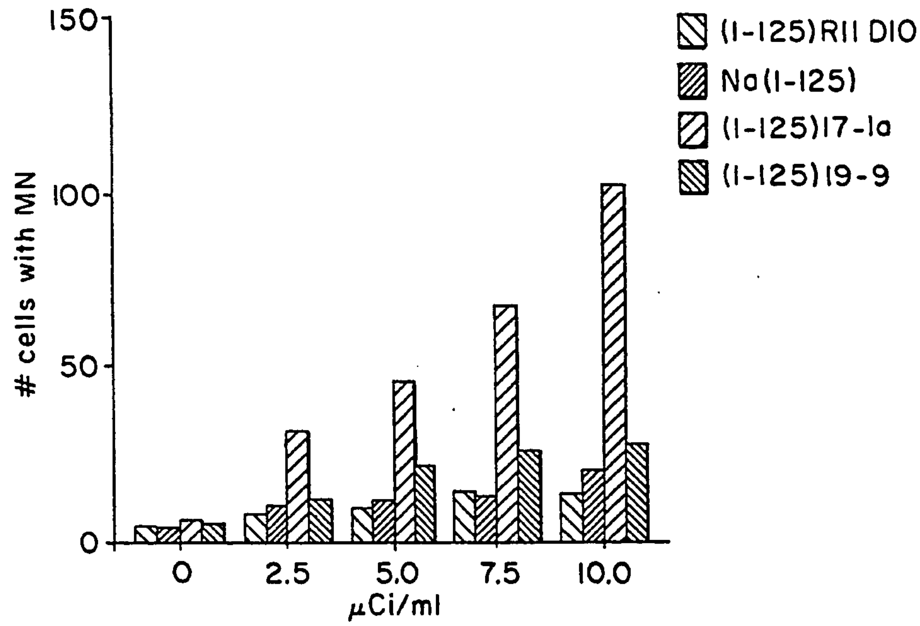

150
100
50
0
cells with MN
0
2.5
5.0
7.5
10.0
μCi/ml
(I-125)RII DIO
Na(I-125)
(I-125)17-Ia
(I-125)19-9

FIG. 4c

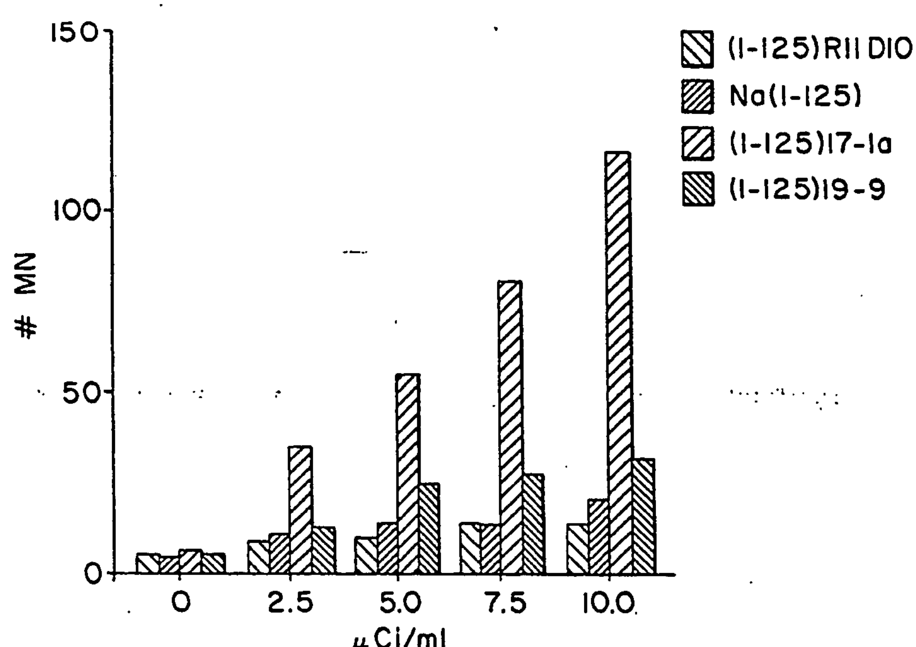

150
100
50
0
MN
0
2.5
5.0
7.5
10.0
μCi/ml
(I-125)RII DIO
Na(I-125)
(I-125)17-Ia
(I-125)19-9

FIG. 4d

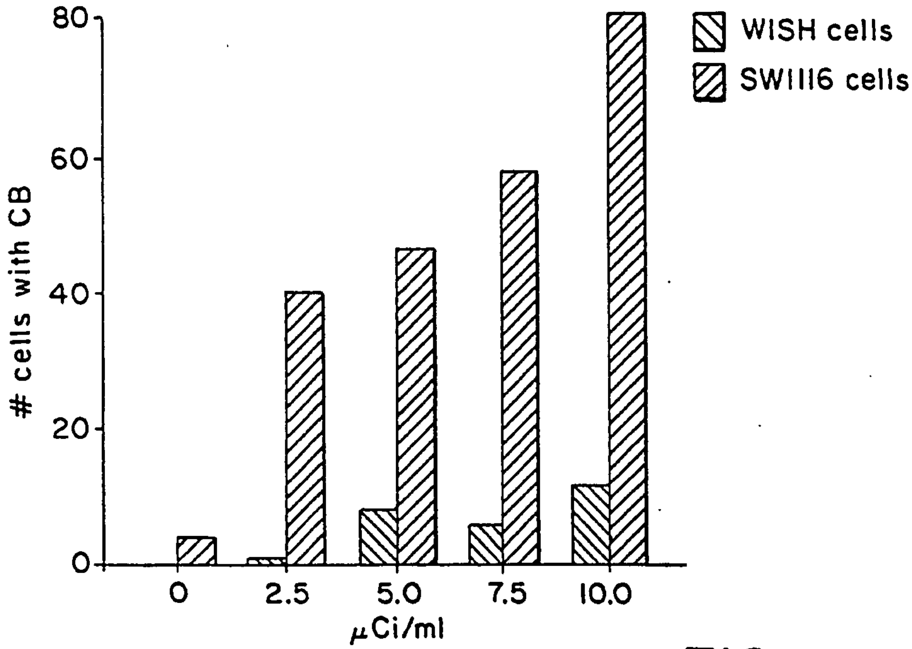

WISH cells
SW1116 cells
cells with CB
80
60
40
20
0
0
2.5
5.0
7.5
10.0
μCi/ml

FIG. 5

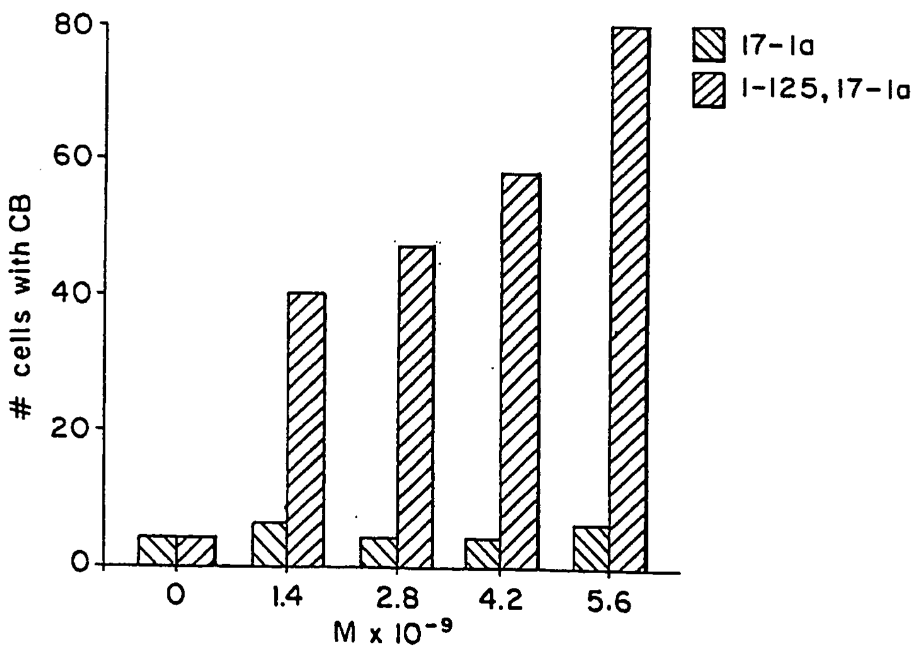
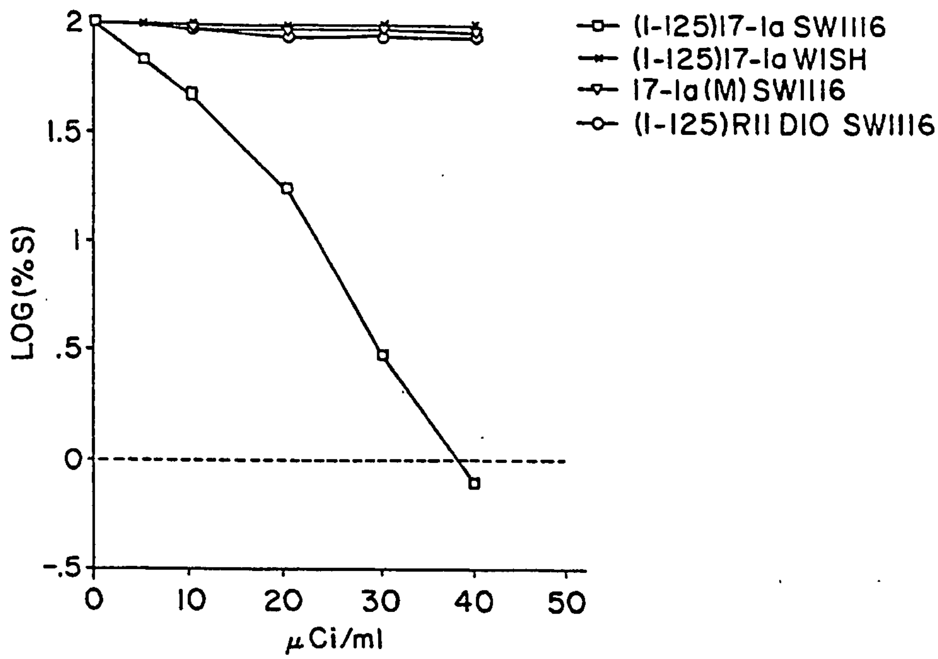

17-1a
I-125, 17-1a
cells with CB
80
60
40
20
0
0
1.4
2.8
4.2
5.6
M x 10^-9

FIG. 6



2
1.5
1
.5
0
-.5
LOG(%S)
0
10
20
30
40
50
μCi/ml
(I-125)17-1a SW1116
(I-125)17-1a WISH
17-1a(M) SW1116
(I-125)R11 D10 SW1116

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/04510

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 47/48, A 61 K 43/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Nucl. Med. Biol. , vol. 16, no. 2, 1989. Intern. Journal of Radiation/Appl. & Instrum. Part B/16, no. 2, 1989 (Marsh Barton, Exeter, GB) G. Mariani et al.: "Monoclonal antibody internalization by tumor cells: an experimental model for potential radioimmunotherapy applications", pages 147-150, see the whole article --	1-3
Y	Somatic Cell Genetics, vol. 5, no. 6, 1979 Plenum Publishing Corp. (GB) H. Koprowski et al.: "Colorectal carcinoma antigens detected by hybridoma antibodies", pages 957-972 see table 2 (cited in the application) --	1-3

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22nd February 1990	29.03.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Cancer Research, vol. 45, October 1985, T.Lindmo et al.: "Specific killing of human melanoma cells by ^{125}I-labeled 9.2.27 monoclonal antibody", pages 5080-5087 see abstract; page 5085, paragraph 9 - page 5086, paragraph 1 (cited in the application)	1-3

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim numbers 4-8., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

Claims 1-3 searched completely
Claims 4-8 not searched, compare PCT Rule 39.1(iv):
Method for treatment of the human or animal body by therapy

3. ☐ Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.